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Structural Elucidation of Urocanate Hydratase Protein Isolated from Salmonella Typhi

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Abstract: The objective of this Study is to elucidate structure of protein urocanate hydratase, docking with suitable drug. The primary concern of modeling a protein is to predict the internal structure, active sites of the protein in order to find out the relevancey to the drug based on various factors such as toxicity, docking score, homology modeling. In this present scenario so there are many kind of sophisticated tools to design any drug. We have selected the disease typhoid fever caused by salmonella typhi the further step is to predict the target molecule (urocanate hydratase) which involves in the typhoid fever. After prediction we have selected template molecules later on superimposing of templates and target molecule, homology modeling, 3 d structure validation, active site prediction, questing for suitable drugs, docking and finally toxicity prediction.

Key words: Docking with suitable drug · Various factors such as toxicity · Typhoid fever caused by salmonella

INTRODUCTION

Typhoid fever is caused by infection of humans with the microorganism *Salmonella enterica* subspecies *enterica* serotype Typhi (*S. typhi* for short). It is a systemic disease characterized by a prolonged fever, malaise and weight loss. On physical examination, characteristic skin lesions, rose spots, usually accompany a hepatosplenomegaly [1]. Without antibiotic treatment the fever may persist for several weeks and the disease will be fatal in about 15 percent of those affected. The bacterium is transmitted by faecal-oral route, through contaminated water or food [2]. *S. typhi* is highly adapted to its human host; there is no reservoir but man. Therefore, every case of typhoid fever means an infection from a previous one [3]. The immunopathogenesis is characterized by a sustained low-grade bacteremia with microbial invasion of and multiplication within the mononuclear phagocytes lining the sinoids of the liver, spleen, bone marrow, lymph nodes and Peyer's patches [4].

The clinical manifestations and severity of disease in typhoid fever may vary widely, largely depending on the patient population, e.g., adults versus infants, studied. Typhoid fever is a disease of children and young adults and most patients who present to hospitals with typhoid fever are in the age class of 5 to 25 years [5]. However, community-based surveillance in high-endemic regions demonstrate that many cases of typhoid, in particular in children under .ve years of age, may have a non-speci.c less severe illness that is not recognized clinically as typhoid [6]. In most developing countries, many patients with typhoid fever do not receive appropriate medical attention or are treated as out patients. The aim of the thesis is to analyse the protein urocanate hydratase protein isolated from salmonella typhi and find the active site and drug binding sites [7].

MATERIALS AND METHODS

Protein Data Bank (pdb): The structural bioinformatics (RCSB)-PDB provides a variety of tools and resources for studying the structure of biological macromolecules and their relationships of sequence, function and disease [8]. This site offers tools for browsing, searching and operating that utilize the data resulting from ongoing efforts to create a more consistent and comprehensive archive [9]. SAVS (structure analysis and verification

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server) is a metaserver for analyzing the protein structures, which is used to check the overall quality factor of a modelled protein [10, 11].

Swiss-pdb viewer is 3 dimensional structure prediction tool which has been developed by Nicolas Guex (GlaxoSmithKline R&D) Swiss Institute of Bioinformatics (SIB) at the structural Bioinformatics Group at the Biozentrum in Basel [12]. The proteins can be superimposed in order to deduce structural alignments and compare the active sites or any other relevant parts. Amino acid H-bonds, angles and distances between atoms are easy to obtain thanks to the intuitive graphic and menu interface [13].

Accelrys Discovery Studio 2.5: Accelrys has released Discovery Studio 2.5, an advanced computational chemistry and biology software environment for drug discovery. Discovery Studio 2.5 streamlines collaboration and increases research productivity, new scientific developments in the area of small molecule modelling, fragment-based design, transmembrane protein analysis and antibody modeling [14, 15]. There is enhanced customisability through new Discovery Identification and prediction of urocanate hydratase and retrieval of urocanate hydratase protein sequence were done by choosing swissprot protein sequence databank to retrieve protein sequence of hydratase urocanate on searching for the typhoid fever protein urocnate hydratase in the site search caption we were granted to retrieve the protein urocanate hydratase from different organism [16]. Out of them we have selected the protein urocanate hydratase isolated form salmonella typi by clicking the switch port Id of the protein urinate hydratase [17]. Once done so the information regarding urocanate hydratase will be displayed. Entry information Name and origin of the protein, protein segment, length of the protein, molecular weight of the protein will be displayed and sequence of urocanate hydratase is retrieved [18, 19].

Secondary Structure Analysis: GOR 4 is one of the proteomic expasy tools which is used to analyse secondary structure of protein. We submitted the protein sequence of urocanate hydratase in protein sequence submission page of GOR 4 secondary structure analysis tools the to GOR 4 tools analysed and predicted the alpha helix, strand, turn, coil, bend region and beta region of urocanate hydratase.

Retrieval of target protein and Tertiary structure prediction (Homology modelling) was carried out by downloading from swiss prot databank [20]. The protein urocanate hydratase was identified and retrieved from swiss prot databank. Swiss prot id HUTU-SALTI, primary accession number is Q8Z897 and enzyme classification numbrber EC 4.2.1.49.

Retrieved protein sequence of urocanate hydratase was submitted in to pair wise sequence alignment tool Protein - protein blast, the blast tool predicted some similar protein sequences which is closely similar with the target protein urocanate hydratase . from the blast result we found that the sequence which 63 % similar to the target protein urocanate hydratase [21]. The selected template protein is isolated from *Bacillus* Sps. The structure of template protein urocanse from bacillus sps is downloaded from protein data bank [22]. The target protein urocanate hydratase is aligned with template protein urocanase isolated from bacillus sps. Identities of target and template is 342/546 (62%) there is no gaps between target and template alignment [23].

Docking using Accelrys Discovery Studio 2.5: Create a new folder and take drug and target protein then load probe and target and change into 100 solutions and then run to differentiate ligand and protein goto view and select molecules [24, 25]. And then go to calculate and analyse it by energy chart. goto animation and start from 80 and end it by 95 and we get best frame in the same way we have to do for all drugs and calculate protein and ligand interaction on the basis of energy graph of all two drugs.

RESULT AND DISCUSSION

In primary structure analysis we have predicted the molecular weight, isoelectric point and physio chemical parameters of the protein urocanate hydratase. We selected expasy proteomic tools to predict the molecular weight isoelectric point and physio chemical parameters of protein urocanate hydratase. We entered in the home page of proteomic expasy tools with help of website addresss is www.expasy.org/tools from the tools list we have chossed primary structre analysis tools are compute PI and protparam. Compute PI tool calculated the molecular weight and iso electric point of the protein urocanate hydratase. The protparam tools calculated amino acids composition, total number of negatively charged amino acids (asp & glu), total number of positively charged amino acids (arg & lys) and stability of protein.

Entry information	
Entry name	HUTU_SALTI
Primary accession number	Q8Z897
Secondary accession numbers	None
Integrated into Swiss-Prot on	August 13, 2002
Sequence was last modified on	March 1, 2002 (Sequence version 1)
Annotations were last modified on	July 22, 2008 (Entry version 44)
Name and origin of the protein	
Protein name	Urocanate hydratase
Synonyms	Urocanase EC 4.2.1.49 Imidazolonepropionate hydrolase
Gene name	Name: hutU OrderedLocusNames: STY0823, t2097
From	Salmonella typhi [TaxID: 601] [HAMAP proteome]
Taxonomy	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Salmonella.
Protein existence	3: Inferred from homology;

>sp|Q8Z897|HUTU_SALTI Urocanate hydratase OS=Salmonella typhi GN=hutU PE=3 SV=1

Fig. 1: Protein sequence of urocanate hydratase

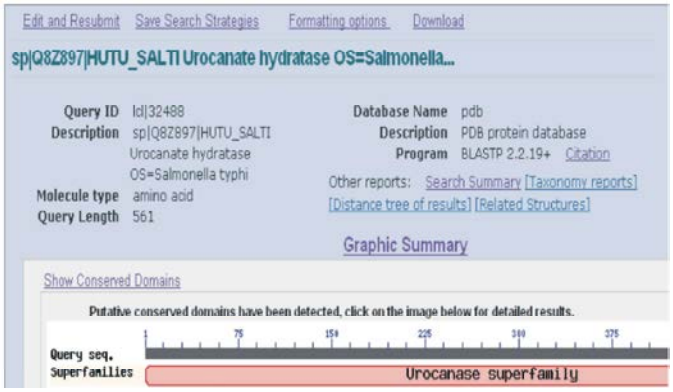
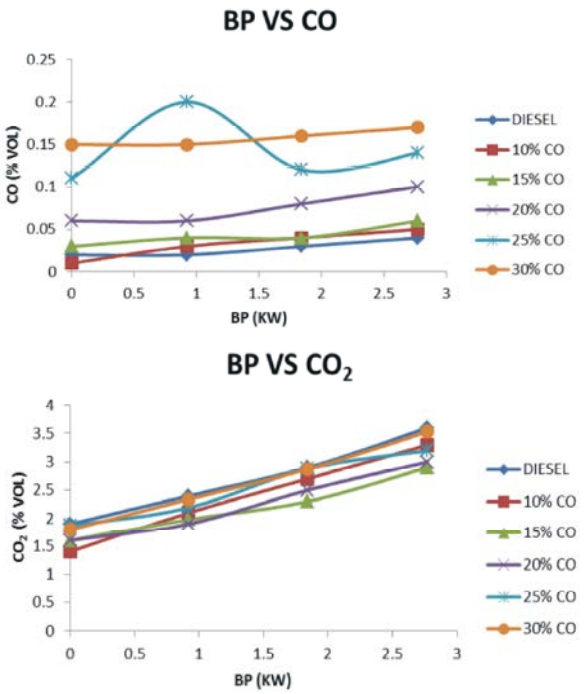
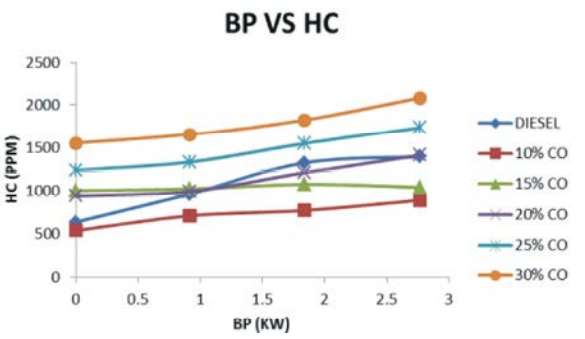


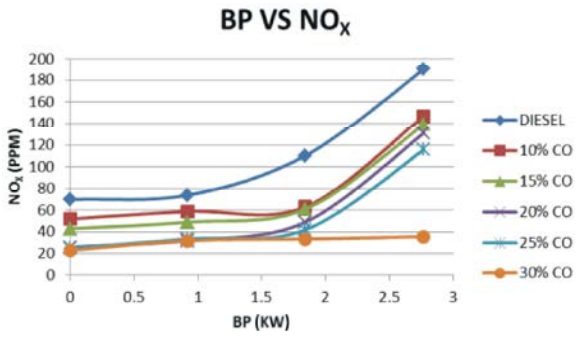
Fig. 2: Identification of template protein structure of urocanse: Chain D, Crystal Structure Of Urocanase From Bacillus Subtilis Length=552

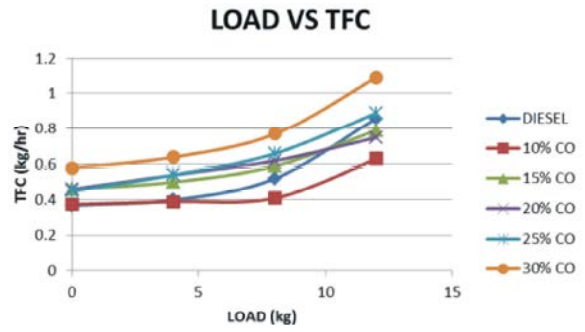
Figure 1 shows the protein sequence of urocanate hydratase of Salmonella typhi retirived from swiss prot protein databank. the amino acids are represented single alphabet codes. Primary accession number of urocanate hydratase in protein sequence swiss prot data bank is Q8Z897 and enzyme classification number is EC.4.2.149. from the result of swiss prot databank we found that there is no structural information about urocanate hudratase of salmonella typhi.

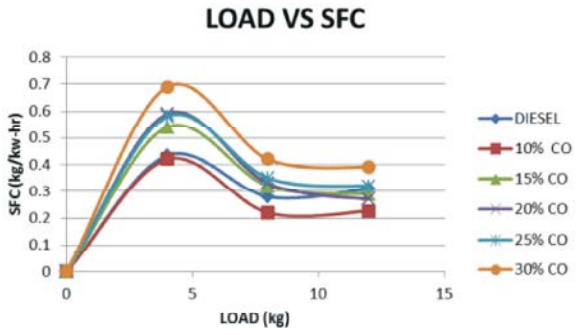
Figure 2 shows the result of protparam proteomic tool. From the result of protparam we found that the Number of amino acids are 561, Molecular weight is 61403.7 Theoretical pI (isoelectric point) is 6.07, amino acid composition, total number of positively charged amino acids, total number of negatively charged amino acids, total number of atoms is 8532, half life of the protein and stability of the protein urocanate hydratase. Our results showed the docking complex of pyridoxal phosphate - urocanate hydratase and the result of Accelrys Discovery Studio 2.5 commercial tool indicated the docking score of pyridoxal is 40.272.

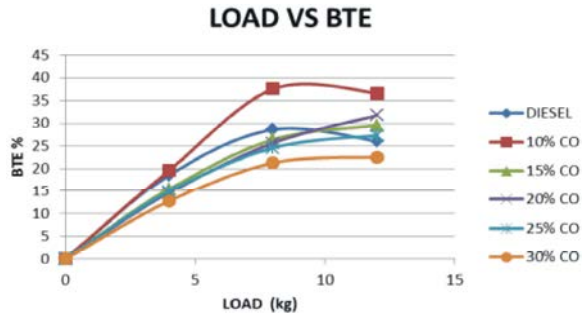












CONCLUSION

The above result shows the structural information of template structure urocanase from bacillus subtilis. Urocanase is retrieved from protein databank and the PDB id is 2fkn. The protein urocanate hydratase from Salmonella typhi have been reviewed. This protein is involved in the disease typhoid and can consider as a drug target and artificial synthesis of this protein can be helpful in the case of vaccine production against this particular epidemic. Three dimensional structure of the urocanate hydratase from Salmonella typhi was elucidated using proteomic tools. Primary structure of urocanate hydratase was predicted using protparam tool, secondary structure predicted using Gor IV tool. And the 3 dimensional structure of urocanate hydratase is prediction using SPBDV offline server. The crystal structure of urocanse from bacillus subitilis was used as the template. The model structure for urocanate hydratase from salmonella typhi is predicted to be good based on the Ramachandran plot. Active site amino acids are anaylsed using Accelrys Discovery Studio 2.5. Finally pyridoxal phosphate and chloramphenical was docked with the modeled protein urocanate hydratase. On comparing the docking score of pyridoxal phosphate and chloramphenical drugs the following results were obtained. Pyridoxal phosphate showed the score 40.272.

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